



Unearthing the species diversity of a cryptozoic snake, *Tantilla melanocephala*, in its northern distribution with emphasis on the colonization of the Lesser Antilles

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Abstract.—*Tantilla* is a diverse New World Colubrid genus comprising 69 small to medium sized, cryptozoic and semi-fossorial species. Morphological data of *Tantilla melanocephala* in the Eastern Caribbean region, and more precisely on the islands of Trinidad and Tobago and nearby Venezuela, have shown differences in scales and color patterns associated with these localities, which may suggest the presence of cryptic species in the region. Assessing the monophyly of *Tantilla melanocephala* is key as its parphyly could compromise important decisions for conservation and management. In this study, we conduct phylogenetic analyses of all available *Tantilla* from GenBank ($n = 11$), including *T. melanocephala* from French Guiana and Brazil, along with novel sequences from Guyana, Venezuela, Trinidad, and Tobago. Broadly, we recover two sister clades within *Tantilla*, a North American-Central American clade and a Central American-South American clade with a time since its most recent ancestor dating to the Mid-Miocene. We found the sampled *T. melanocephala* to be monophyletic in all analyses and estimated the origin of this clade towards the early Pleistocene. The close association between Trinidad and Venezuela, dating towards the end of the Pleistocene, suggests connections in the recent past. This study is the first to assess the intraspecific variation in the species and we hope it will set a landmark to complete the systematic characterization of the entire species throughout its widespread distribution.

Keywords. Biogeography, colonization, dispersal, Reptilia, Tobago, Trinidad

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Introduction

Despite the widespread distribution ranges of certain cryptic species, their presence on islands often reveals lineages divergent from those on the mainland and even the presence of new species (Card et al. 2016; Jowers et al. 2019; Murphy et al. 2019a,b). Therefore, an understanding of both the ecology and evolutionary history of an endangered species on islands are pivotal for ensuring their effective protection and conservation (Young 2000; Spielman et al. 2004). The Trinidad and Tobago Archipelago is located on the continental shelf

of northern South America with the Caribbean Sea to the north, the Atlantic Ocean to the east, and the Gulf of Paria to the west. It is composed of two main islands (Trinidad and Tobago) and about 20 smaller satellites and offshore rocks. While both larger islands are considered the southernmost Lesser Antilles, they have a continental flora and fauna with two distinctly different geological origins. Trinidad was previously attached to Venezuela and formed by a pull-apart basin in the Late Miocene, when a downward warping event separated both land masses (Liddle 1946; Erlich and Barrett 1990). Tobago, on the other hand, was formed as an oceanic island on

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the front edge of the Caribbean Plate far to the West of its current position (Pindell and Kennan 2007; Jowers et al. 2015). These islands can be considered as an eastward extension of the sky island complex formed by the Venezuelan coastal ranges. Their geographic position is also unique because they lie just to the north of the Guiana Shield at the mouth of the Orinoco River (Fig. 1).

Widespread taxa that span across biogeographic barriers can pose a particular challenge for taxonomists as isolation processes can often lead to the presence of cryptic lineages. Barriers to gene flow allow opportunities for speciation, and their removal offers the opportunity for secondary contact that may result in introgression. Species that are widely distributed are also dispersing from multiple distant locations, and genetic material from these populations could converge at more proximate geographic localities with the assistance of wind or water (Reynolds et al. 2020). Furthermore, these species are key for understanding how diverse ecological factors may drive regional patterns of species divergence and speciation (Card et al. 2016). Fortunately, advances in molecular phylogenetics can provide resolution to our understanding of the evolutionary history of even rarely studied species.

The black-headed snakes of the genus *Tantilla* are small to medium sized (usually < 300 mm) Western Hemisphere snakes that specialize in feeding on arthropods, particularly centipedes (Marques and Puerto 1998). Currently, the genus comprises 69 species (Uetz et al. 2020) distributed from sea level to at least 3,000 m and ranges from Nebraska (USA) to Santa Fe Province (Argentina). It is present on both the Pacific and Atlantic versants from Guatemala southward through Central America into South America, reaching as far south as southern Peru, Bolivia, northern Argentina, and Uruguay; and it is also present in the Lesser Antilles, on the Trinidad and Tobago islands (Henderson and Powell 2006, 2018). *Coluber melanocephala* Linnaeus (1758) was the first member of the genus described, and it has

been re-described at least ten times since Linné's original description (Wilson and Mena 1980). The distribution of *T. melanocephala* as currently understood covers much of the Neotropics, from Colombia to northern Argentina and Uruguay, including the islands of Trinidad and Tobago (Fig. 1). Recently, this species was recorded on St. Vincent and Grenada banks, where its presence has been documented since at least 2005, and it presumably arrived through human-mediated introduction in construction material from Guyana and probably also from Trinidad and Tobago (De Silva and Wilson 2006; Henderson and Powell 2006, 2018; Berg et al. 2009; Henderson and Breuil 2012). Records from Panama correspond to misidentifications of *T. armillata* (Ray 2017). Like some congeners, it is also present on both sides of the Andes. Greenbaum et al. (2004) synonymized *T. equatoriana* Wilson and Mena 1980 with *T. melanocephala* based on a morphometric data principal component analysis.

The phylogenetic position of *Tantilla* remains inconclusive as several molecular phylogenetic studies have found alternative placements of the genus among the Colubridae (Pyron et al. 2013; Jadin et al. 2014; Koch and Venegas 2016; Figueroa et al. 2016; Zaher et al. 2019). Pyron et al. (2013) used only *T. melanocephala* as a representative for the genus in their squamate study, finding *Tantilla* and *Salvadora mexicana* to be the sister to *Coluber* and *Masticophis*. This generic placement of *Tantilla* contrasted with Jadin et al. (2014) who found *T. relictata* as sister to *Conopsis*, *Pseudoficimia*, and *Sympholis*. However, both of these relationships were later confirmed by Figueroa et al. (2016) who included more *Tantilla* taxa and found the genus to be paraphyletic, reached similar conclusions to Pyron et al. (2013) and Jadin et al. (2014), and consequently considered its placement in the larger snake phylogeny unresolved. Koch and Venegas (2016) included *T. amilatae*, *T. impensa*, *T. melanocephala*, and *T. vermiformis* in their description of *T. tjiasmantoi* but had no support for intergeneric

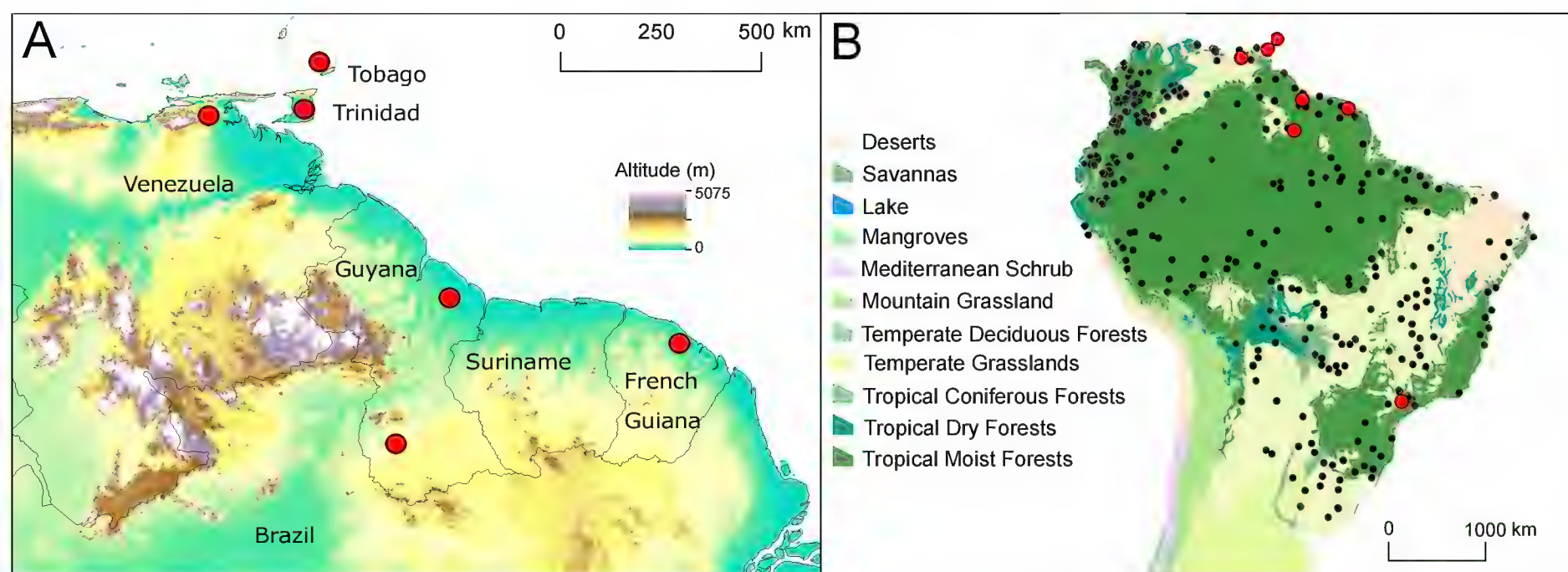


Fig. 1. (A) *Tantilla melanocephala* sample localities for this study (red circles) in the northern region of its distribution. (B) The distribution of *Tantilla melanocephala* in the Neotropics. Locality data are from VertNet and the GBIF databases, as well as the literature (Nogueira et al. 2019). Within the Lesser Antilles, Union Island and the Mustique islands are not shown. Red circles are *T. melanocephala* localities included in the phylogenetic analyses. The map suggests this species inhabits several different biomes.

relationships. Zaher et al. (2019) reported *T. relicta* and *T. melanocephala* as being sister to *Scolecophis*, and together they were sister to a clade of colubrids consisting of *Conopsis*, *Ficimia*, *Gyalopion*, *Pseudoficimia*, *Sympholis*, *Sonora*, and *Stenorrhina*.

Along with *Boa constrictor*, *Tantilla melanocephala* is among the most widely distributed snakes in South America. *Tantilla melanocephala* is distinguished from congeners by the following combination of traits: (1) dark head cap transitions into dark nape band with two pale spots covering the posterior parietals, posterior edge of temporals, and associated post parietal scales; (2) pale preocular spot; (3) lateral extension of the head cap contacts gulars; (4) no pale nape band posterior to the dark nape band; (5) background color tan with nine dark brown stripes; and (6) a greater number of subcaudals compared to other members of the *T. melanocephala* group. *Tantilla melanocephala* is characterized by extensive geographic variation in the color pattern, a wide range of ventral (126–177 ventrals) and subcaudal counts (41–74), as well as occupation of seven different biomes (Wilson and Mena 1980; Wilson 1992). Several attempts to clarify the marginal populations have been conducted superficially based in their morphology, mainly on color patterns and cephalic scutellation (Wilson and Mena 1980; Vuoto 1998). Wilson and Mena (1980) discussed the variation of *T. melanocephala* ventral scale counts in two Caribbean islands, noting that the differences in ventral counts between specimens from Trinidad, adjacent Venezuela, and Tobago, are striking (Fig. 2). Similarly, the authors found significant differences in subcaudals between mainland Venezuela and the islands. In addition, six color patterns were detected across the range in *T. melanocephala* by Wilson and Mena (1980), with Trinidad and Tobago specimens expressing two of these patterns along with specimens from Argentina, Colombia, Guyana, Paraguay, Suriname, Uruguay, and Venezuela.

Herein, we assess whether populations from the islands of Trinidad and Tobago, and proximal mainland localities of Venezuela (Peninsula de Paria) and Guyana, constitute the same lineage or if they are part of a cryptic lineage complex. Furthermore, molecular phylogenetics are used to examine evolutionary relationships within *Tantilla*. Finally, the likely time of colonization from the mainland is explained, along with colonization of the islands in relation to climatic conditions in the region. Given the morphological variation between the two island populations that are now separated by 50 km of water and were once connected to each other at glacial maxima when sea levels had dropped, the genetic distance between the two populations is investigated.

Materials and Methods

Tantilla melanocephala specimens were collected from locations in Trinidad, Tobago, and Venezuela, under licenses from the Trinidad and Tobago Government



Fig. 2. Specimens of *Tantilla melanocephala* from (A) Tobago, Pigeon Point, (B) Trinidad, Bush Bush, Nariva Swamp, and (C) Venezuela, Caracas, Distrito Capital. Photos by J.C. Murphy (A–B) and L.A. Rodríguez (C).

Wildlife Section: Special Game Licenses issued for scientific purposes in 2015–2016 to John Murphy, Renoir Auguste, and Mike Rutherford; and under collection permit number 1,375 granted to Gilson A. Rivas by the Ministerio del Poder Popular para Ecosocialismo y Aguas, Venezuela. Animals were euthanized following the ASIH guidelines (Beaupre et al. 2004) using pentobarbital. Museum acronyms follow Sabaj (2019).

DNA was extracted using a Qiagen DNeasy blood and tissue kit (Qiagen, Hilden, Germany) following the instructions of the manufacturer (see Supplemental Table S1 for list of primers). The target genes were the mitochondrial small and large ribosomal subunits (12S

rDNA and 16S rDNA, respectively), cytochrome *b* (*cytb*) and the nuclear oocyte maturation factor (*c-mos*; see Supplemental Table S2). Sequence editing follows Murphy et al. (2019c). Despite some individuals having different lengths in some alignments, the lengths of the alignments were: 12S rDNA, 404 base pairs (bp); 16S rDNA, 494 bp; *cytb*, 1,086 bp; and *c-mos*, 561 bp. *Cytb* and *c-mos* were translated into amino acids to check for the presence of stop codons. Following Jadin et al. (2014, 2019), Figueroa et al. (2016), and Zaher et al. (2019), all genera that were sister to *Tantilla* were included, while *Drymarchon couperi* and *D. corais* were used as outgroup (Supplemental Table S2). Sequences were aligned in Seaview v4.2.11 (Gouy et al. 2010) under ClustalW2 default settings (Larkin et al. 2007) for the *cytb* and *c-mos* fractions, and with MAFFT (Kato et al. 2002) for the 12S and 16S rDNA. The 12S and 16S rDNA, *cytb*, and *c-mos* sequences were concatenated, resulting in a 2,548 bp alignment.

Phylogenetic analyses were performed using the Bayesian Inference and Maximum Likelihood methods. MrBayes v3.2 (Ronquist and Huelsenbeck 2003) was used to construct the Bayesian Inference tree under the best-fitting substitution model for each gene partition. ML searches were conducted in RAxML v7.0.4 (Silvestro and Michalak 2012), using partition data sets under default settings, and support was assessed by using 1,000 bootstrapped replicates. The most appropriate substitution model was implemented for each gene fragment as determined by the Bayesian Information Criterion in PartitionFinder v2 (Lanfear et al. 2017) to choose the optimal partitioning strategy for both phylogenetic analyses (Supplemental Table S3). Default priors and Markov chain settings were used, and searches were performed with random starting trees. Each run consisted of four chains of 20 million generations, sampled every 2,000 generations.

BEAST v1.8.4 (Drummond et al. 2012) was used to simultaneously estimate the phylogeny and divergence times between taxa. The most appropriate substitution model was implemented for each gene fragment as determined by the Bayesian Information Criterion in jModeltest v2 (Posada 2008). A Yule speciation tree prior was applied, along with a relaxed lognormal clock for the concatenated 12S+16S rDNA and for the *c-mos* alignments. A strict clock, using a substitution rate of 1.34% substitutions per million years, was applied for the *cytb* gene fragments, as estimated by Daza et al. (2009) for Neotropical colubrids based on four calibration points. As priors for the rates, the approximate reference (CTMC) prior was selected (Ferreira and Suchard 2008). BEAST was run twice with 50 million generations per run, sampling every 5,000 steps. Convergence of the runs was verified in Tracer v1.6 (Rambaut et al. 2013), both runs were combined in LogCombiner, and the Maximum Clade Credibility Tree was computed using Tree Annotator (BEAST v1.8.4). All analyses were performed

through the CIPRES platform (Miller et al. 2010).

Results

No stop codons were found in the *cytb* and *c-mos* alignments. The best-fitting models are shown in Supplemental Table S3. All phylogenetic analyses recovered *Tantilla melanocephala* as monophyletic (Figs. 3–4). Similarly, all analyses recovered a strongly supported *T. melanocephala* from Venezuela+Trinidad clade. The relationship with Tobago is weakly supported (Fig. 3). The timing of the most recent common ancestor of *Tantilla* dates to the Middle Miocene (~12 Mya: 95% HPD 10.7–14.3 Mya), but this time estimate is likely to change when other species are included in future phylogenetic analyses (Fig. 4). Timing of the *T. melanocephala* clade varies considerably, with the time since the most recent common ancestor (TMRCA) dating to the beginning of the Pleistocene (2.3 Mya: 95% HPD 1.8–3 Mya). This early split relates to the timing between *T. melanocephala* from Brazil and all other localities of *T. melanocephala*. The divergence between Guyana + French Guiana and Venezuela + Trinidad + Tobago dates to 1.8 Mya (95% HPD 1.2–2.4 Mya) (Fig. 4). A more recent TMRCA towards the end of the Pleistocene is recovered between Trinidad and Venezuela *T. melanocephala* (0.2 Mya, 95% HPD 0.014–0.48 Mya). The recovered sister clade relationship between *T. tjiasmantoi* (from Peru) and *T. melanocephala* requires further investigation as *T. tjiasmantoi* is the only *Tantilla* species missing *cytb* and *c-mos* sequence data.

Discussion

The analyses presented here recovered two clades within *Tantilla*, a North American-Central American clade consisting of *T. coronata*, *T. gracilis*, *T. hobartsmithi*, *T. impensa*, *T. nigriceps*, *T. planiceps*, and *T. relictata* and a Central American-South American clade with *T. armillata*, *T. vermiformis*, *T. tjiasmantoi*, and *T. melanocephala*. The calibration estimates for *T. melanocephala* divergence reject a vicariant event between Trinidad and northern South America in the Late Miocene when Trinidad detached from the Peninsula de Paria of northern Venezuela ~4 Mya (Babb and Mann 1999; Flinch et al. 1999), and point to a divergence in the Late Pleistocene. The Late Pleistocene was a time of rapid and abrupt topographic change in the eastern Caribbean associated with eustatic sea level changes in the region (Murphy 1997; Hansen et al. 2013; Murphy et al. 2019c). The low genetic divergence of *T. melanocephala* recovered between Venezuela and Trinidad is similar to that found in *Atractus trilineatus* between Guyana and the islands (Murphy et al. 2019c), and it likely results from the changing topographical conditions that facilitated the connections between regions (Murphy 1997; Murphy et al. 2019c). Different parts of the Trinidad and Tobago

Tantilla melanocephala diversity in Trinidad, Tobago, and Venezuela

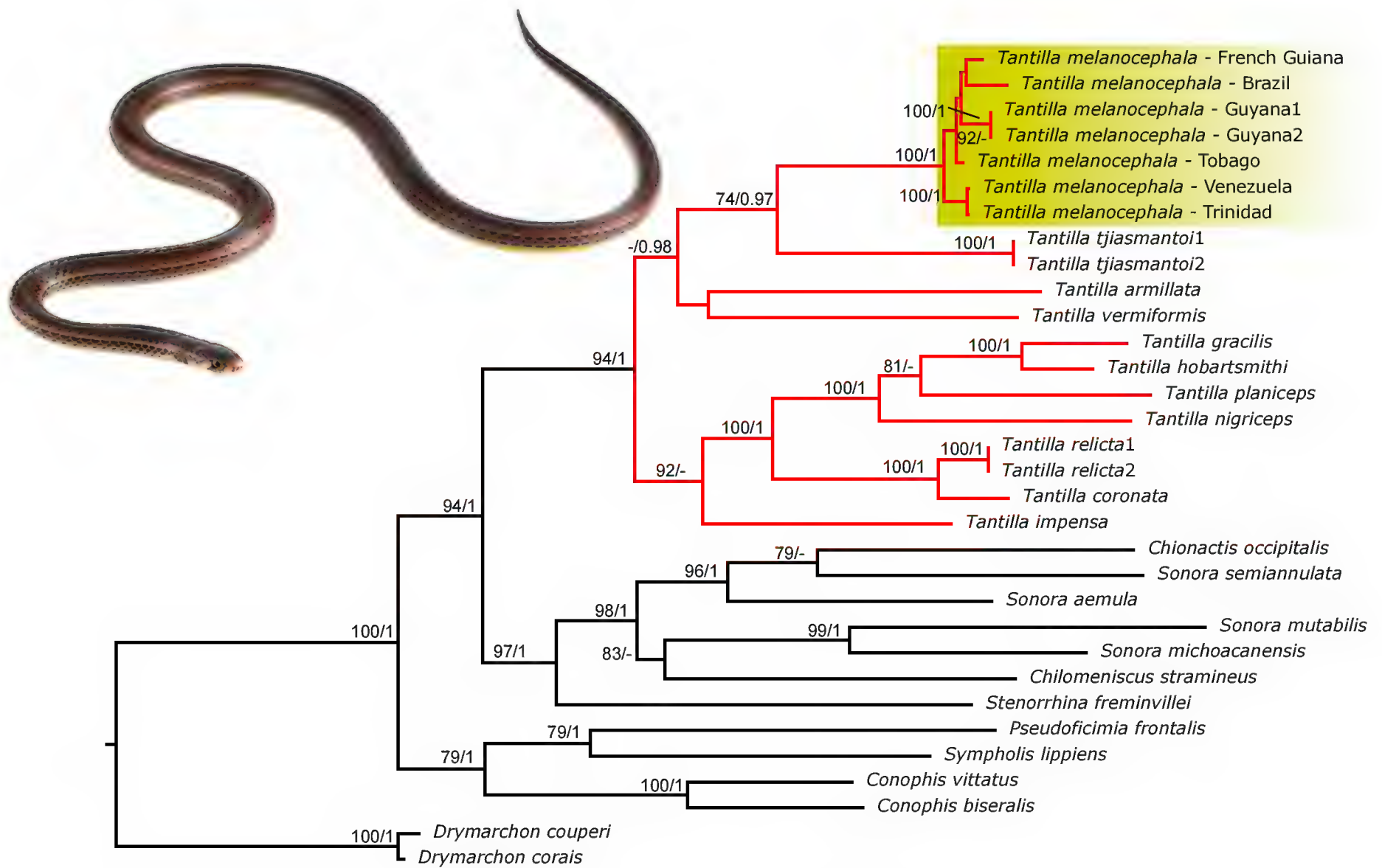


Fig. 3. Best Maximum Likelihood tree based on the data set of concatenated 12S and 16S rDNA, cytb, and c-mos sequences. Red clade depicts the genus *Tantilla*. Values on the left and right sides of a slash (/), are the values indicated at nodes of Maximum Likelihood bootstraps (>70%) and Bayesian Posterior probability values (>95%), respectively. The *Tantilla melanocephala* pictured is from the western versant of the Occidental slopes in Ecuador (from the Rio Manduriacu Reserve). *Photo by R. Maynard.*

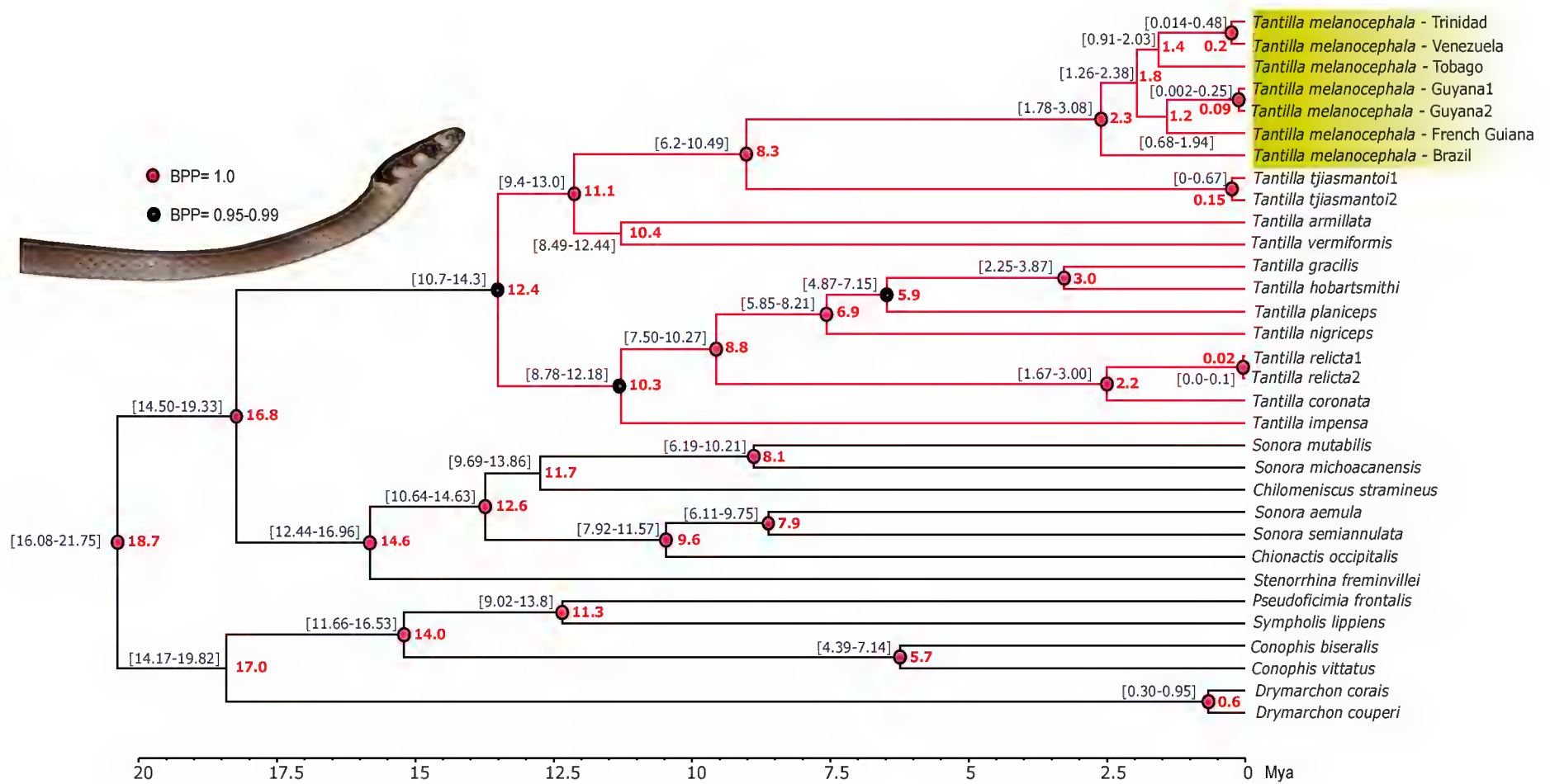


Fig. 4. Bayesian time tree as inferred by BEAST for the data set of concatenated 12S and 16S rDNA, cytb, and c-mos sequences from *Tantilla* specimens (in red). Red values by nodes denote the median time estimates, whereas values in brackets denote 95% Highest Posterior Density ranges. Red and black nodes are posterior probabilities (1.00 and >95–99%), respectively. *Photo by J.C. Murphy.*

archipelago were connected to the mainland multiple times, with Trinidad connected to the mainland for more prolonged periods than Tobago (Murphy et al. 2019c).

When sea levels dropped during the Pleistocene, gene flow could be expected between island populations and the mainland, as connections could be established with sea level drops as moderate as 50 m (Murphy 1997; Hansen et al. 2013). Thus, recent divergences in a variety of organisms were likely. For example, Robinson's Mouse Opossum (*Marmosa robinsoni*) from Venezuela and Trinidad and Tobago has been shown to diverge 0.34 Mya (Gutierrez et al. 2014), and the skinks *Marisora aurulae* from Trinidad and *M. falconensis* from Estado Falcón in the Paraguaná Peninsula, diverged ca. 0.5 Mya (Hedges and Conn 2012). Therefore, vicariance is more plausible than an over-water dispersal during the interglacial periods. Nevertheless, over-water dispersal cannot be ruled out as an explanation for the arrival of species in Tobago from Trinidad (e.g., Boos 1984a,b; Camargo et al. 2009; Murphy et al. 2016). For example, the shared *Micrurus diutius* haplotypes in Trinidad and Guyana and the low divergence of *Atractus trilineatus* from Trinidad, Tobago, and Guyana suggest dispersal through either Pleistocene land bridge formations or rafting (Murphy et al. 2019c).

Furthermore, recent work on the fossorial Trinidad Threadsnake, *Epictia tenella*, has shown a remarkable genetic similarity between Guyana and Trinidad, suggesting colonization by recent dispersal rather than ancient vicariance (Murphy et al. 2016). The results of the current study indicate that mainland southern localities (Guyana and French Guiana) show deeper divergence compared to Venezuela-Trinidad and Tobago, with a TMRCA dating to circa 1.8 Mya and a basal split between Brazil and the rest of the clade dating to 2.3 Mya. Similarly, the frog *Leptodactylus validus* originates from northern South America (Guyana/Brazil) and its dispersal to Trinidad ~1 Mya has been attributed to periods of intermittent land connections, or overwater dispersal, throughout the Pleistocene (Camargo et al. 2009). This pattern was also reported for *Atractus trilineatus* between individuals from coastal Guyana and Trinidad-Tobago (Murphy et al. 2019c). Indeed, dispersals from the northern mainland to Trinidad may be a regular occurrence and a source of close genetic associations with local herpetofaunal populations (Charles 2013). More effective conservation measures can thus be implemented based on increased knowledge of the distribution and systematics of relevant species (Bohm et al. 2013).

The recovery of a strongly monophyletic clade contrasts with what we might have expected based on morphological data (Wilson and Mena 1980). Combining data from Wilson and Mena (1980) and the data presented here, the mean ventral counts on Trinidad males (146.8) are lower by approximately 5.8 scales than on animals from adjacent Venezuela (where males average 152.6);

for females the difference is even greater at 9.3 scales (Trinidad averages 150.7 ventrals and adjacent Venezuela averages 160). Comparing Tobago and Trinidad, male ventral numbers again increase markedly in Tobago over those in Trinidad, and are greater than those from mainland Venezuela. Wilson and Mena (1980) also commented that only specimens from upper Central America have higher ventral counts than those from Tobago. Comparable figures for Tobago females were not available, but the count for the single Tobago female (168) was nine scales more than the highest count for Trinidad females. In contrast, subcaudal counts were higher in Venezuela (male average 66.4) and Trinidad (male average 57.2), but subcaudal counts in Tobago males averaged 73.25. Additional population sampling will be required from marginal populations (such as those in Argentina, Uruguay, southern Brazil, and west of the Andes) to encompass the wider distribution of the species and ascertain the complete range of morphological variation in the species.

Our phylogenetic analyses provide evidence for the idea that at least a portion of the Trinidad and Tobago herpetofauna has closer ties to the Caribbean Coastal Ranges (CCR) of Venezuela than to the Guiana Shield. Distribution patterns and molecular evidence from other taxa, such as various frogs (*Flectonotus fitzgeraldi*, *Hyalinobatrachium orientale*, *Mannophryne olmonae*, *M. trinitatis*, *Pristimantis charlottevillensis*, *P. turpinorum*); lizards (*Anolis* cf. *tigrinus*, *Bachia trinitatis*, *Gonatodes ceciliae*, *G. ocellatus*, *Oreosaurus shrevei*, *Plica caribeanus*, *Polychrus auduboni*); and snakes (*Atractus fuliginosus*, *Dipsas variegata*, *Erythrolamprus bizona*, *E. pseudoreginae*, *Ninia atrata*, *Micrurus circinalis*), corroborate a shared fauna between Trinidad, Tobago, and the CCR (Murphy 1997; Angarita-Sierra 2014; Jowers et al. 2015; Murphy et al. 2018). However, *Tantilla melanocephala* is a widespread species that also has a shared genetic history with the CCR despite its proximity to the Guiana Shield. We suspect that as more species are examined this pattern will become more prevalent.

Conservation

The total number of *Tantilla melanocephala* individuals observed on Trinidad and Tobago over 15 field trips was only 12 (JCM field notes). In contrast, Lynch (2015) found *T. melanocephala* to be among the five most frequently encountered snakes in African Oil Palm plantations that were sampled in the department of Meta, Colombia. However, *T. melanocephala* was among the rarest snakes found in oil palm plantations at all other sites sampled across Colombia. Such findings suggest that *T. melanocephala* might be associated with certain habitat types or dynamics (e.g., oil palm plantations surrounded by forest as opposed to pasture), being very common in some areas or very rare in others. However, it

is also plausible that the *T. melanocephala* data reported by Lynch (2015) are representative of more than one lineage, suggesting that some populations are more tolerant of disturbed habitats than others.

Our study shows the lack of cryptic species diversity within a few regions of the peripheral populations of *T. melanocephala*. This finding implies that (at least for now) its conservation status of Least Concern (IUCN 2020) is suitable. Increasing the collection and sequencing efforts across most of the distribution range of this species will be challenging, but such an effort would likely address the presence of population diversity and morphotypes from different habitats. In particular, sampling of biogeographically important regions (e.g., Trans-Andean) might reveal divergent lineages that warrant a more protective conservation status.

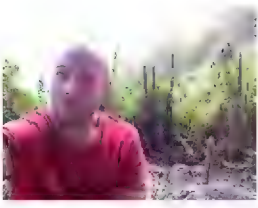
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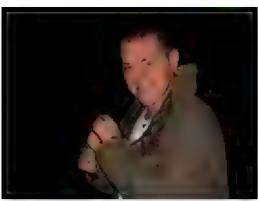
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Michael J. Jowers is an evolutionary biologist with broad interests in the processes and the timings of speciation. His work focuses on tropical island biogeography, phylogeography, systematics, population genetics, taxonomy, and conservation. Michael is deeply involved in amphibian and reptile studies from the islands of Trinidad and Tobago (Lesser Antilles), but he is also interested in other organisms such as birds, mammals, and insects, and actively leads studies throughout South America, Africa, Europe, and Asia.



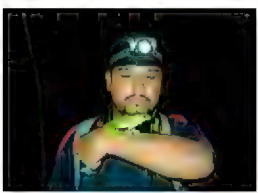
Gilson A. Rivas was born in Caracas, Venezuela. He currently serves as co-editor of the scientific journal *Anartia*, and is a collection manager at the Museo de Biología de la Universidad del Zulia, Maracaibo—a Venezuelan centennial university that began academic activities on 11 September 1891. For over two decades, Gilson has been devoted to the taxonomy and conservation of the neotropical herpetofauna, having authored or co-authored more than 100 academic publications, describing over 30 new species of amphibians and reptiles, and a new genus of dipsadine snake, *Plesiodipsas*. Gilson is the author (with G. Ugueto) of the book *Amphibians and Reptiles of Margarita, Coche, and Cubagua*; and together with M. De Freitas, H. Kaiser, C.L. Barrio-Amorós, and T.R. Barros produced *Amphibians of the Península de Paria: a Pocket Field Guide*. Gilson's research interests are focused on the herpetofauna of the Venezuelan coastal range and insular ecosystems, as well as the influences of invasive species and human development and their impacts on the native fauna.



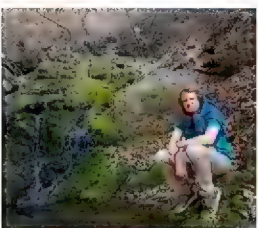
Robert C. Jadin is a lecturer and curator at the University of Wisconsin, Stevens Point (USA). Robert completed his Ph.D. from the Department of Ecology and Evolutionary Biology at the University of Colorado, Boulder, after transferring from the University of Texas at Arlington. Robert's specialties are biodiversity informatics, herpetology, and systematics, and he typically works on snakes. More specifically, his research encompasses bioinformatic and comparative approaches to address evolutionary and biodiversity questions about species relationships, distributions, adaptations, and conservation implications. In this photo, Robert holds a *Sibon longifrenis* at La Selva, Costa Rica. *Photo by S.A. Orlofske.*



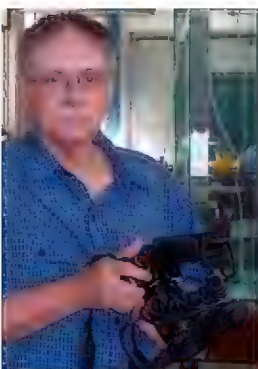
Alvin L. Braswell has a B.S. in Wildlife Biology and an M.S. in Zoology with an emphasis in Herpetology. He retired after 40 years with the Museum of Natural Sciences (MNS, Raleigh, North Carolina, USA) where he transitioned thru the ranks as Collections' Manager for Herpetology and Ichthyology, Curator of Amphibians, Curator for Herpetology, Research Lab Director, and finally Deputy Museum Director for Operations. After 4.5 years of retirement, the joy of being a biologist again, and discovering new and wonderful things, he was called back to serve as Interim Director of the MNS. Now (a year and half later), he is looking forward to re-retirement and having the chance to be a biologist again. Alvin holds an adjunct position at North Carolina State University, where he has co-taught Herpetology from 1996–2013.



Renoir J. Auguste is a Trinidad and Tobago herpetologist. Renoir received his M.Sc. in Biodiversity Conservation from The University of the West Indies, St. Augustine Campus, Trinidad and Tobago, and is interested in the ecology and conservation of amphibians and reptiles. He has conducted herpetological surveys across Trinidad and Tobago for national baseline surveys aimed at improving protected areas, as part of his academic degrees, and also voluntarily with the local environmental NGO *Trinidad and Tobago Field Naturalists' Club*, in which he has held the position of President for three years.



Amaël Borzée is principally interested in behavioral ecology and the conservation of species; and his current research focus is on amphibian breeding behavior and conservation in North East Asia. Amaël has been mostly focusing on treefrogs so far, but is currently expanding his interests to address broader questions for the conservation of multi-species populations over large landscapes, including the use of multiple types of approaches and analytical tools.



John C. Murphy is a naturalist with a focus on snakes. When he is not hiking in the desert or examining specimens in the lab, he is often writing about reptiles. Murphy is a retired science educator who got serious about his lifelong fascination with lizards and snakes in the early 1980s when he and his family made their first trip to Trinidad. The work on Trinidad and Tobago provided valuable lessons that shaped his views of nature and evolution, and today he is still working on the eastern Caribbean herpetofauna. In the 1990s he did some work on homalopsid snakes in Southeast Asia with others from the Field Museum (Chicago, Illinois, USA). He now resides in southeastern Arizona, and is involved in multiple projects on arid habitats and the impacts of climate change on biodiversity. His most recent book is *Giant Snakes, A Natural History* (with co-author Tom Crutchfield). Born and raised in Joliet, Illinois, he first learned about reptiles on his grandfather's farm by watching Eastern Garter Snakes emerge from their winter dens and Snapping Turtles depositing their eggs at the edge of a cattail marsh.

Supplementary Material

Table S1. Primers used in gene fragment amplification, indicating the gene fragments amplified, primers, and references.

Gene	Primer name and sequence	Reference
12S rDNA	12SA 5'-AAACTGGGATTAGATACCCCACTAT -3'	Kocher et al. 1989
12S rDNA	12SB 5'-GAGGGTGACGGGCGGTGTGT -3'	Kocher et al. 1989
16S rDNA	16SL 5'-GCCTGTTTATCAAAAACAT -3'	Palumbi et al. 1991
16S rDNA	16SH 5'-CCGGTCTGAACTCAGATCACGT -3'	Palumbi et al. 1991
cytb	14910 5'-GACCTGTGATMTGAAAAACCAAYCG -3'	Burbrink et al. 2000
cytb	H16064 5'-CTTTGGTTTACAAGAACAATGCTT -3'	Burbrink et al. 2000
cytb	Pacek-L (short) 5'-TGAGGACAAATATCATTCTGAGG -3'	Ptacek et al. 1994
cytb	CB3Xen-H 5'-GGCGAATAGGAARTATCATTC -3'	Goebel et al. 1999
c-mos	S77 5'-CATGGACTGGGATCAGTTATG -3'	Lawson et al. 2005
c-mos	S78 5'-CCTTGGGTGTGATTTTCTCACCT -3'	Lawson et al. 2005

Table S2. Species used in this study, vouchers, and GenBank accession numbers. na: not applicable; asterisks (*) indicate locality identity not confirmed.

Species	Ingroup vouchers	Ingroup country	12S rDNA	16S rDNA	Cytb	C-mos
<i>Chilomeniscus stramineus</i>	na	na	--	--	GQ895856	GQ895800
<i>Chionactis occipitalis</i>	na	na	--	--	GQ895857	GQ895801
<i>Conophis vittatus</i>	na	na	--	--	GQ895862	GQ895806
<i>Conopsis biserialis</i>	na	na	--	--	GQ895860	GQ895804
<i>Pseudoficimia frontalis</i>	na	na	--	--	GQ895886	GQ895827
<i>Sonora aemula</i>	na	na	--	--	JQ265959	JQ265952
<i>Sonora michoacanensis</i>	na	na	--	--	JQ265958	JQ265951
<i>Sonora mutabilis</i>	na	na	--	--	JQ265956	JQ265950
<i>Sonora semiannulata</i>	na	na	--	--	AF471048	AF471164
<i>Stenorrhina freminvillei</i>	na	na	HM565769	--	GQ895889	GQ895830
<i>Drymarchon corais</i>	na	na	HM565758	HM582218	AF471064	AF471137
<i>Drymarchon couperi</i>	na	na	--	--	KP765662	KP765646
<i>Sympholis lippiens</i>	na	na	--	--	GQ895890	GQ895831
<i>Tantilla coronata</i>	LSU H-18896	USA	--	--	KP765669	KP765653
<i>Tantilla gracilis</i>	OMNH41880	USA	--	--	KP765670	KP765654
<i>Tantilla hobartsmithi</i>	MVZ233299	USA	--	--	KP765671	KP765650
<i>Tantilla planiceps</i>	TAPL340	USA	--	--	KP765673	KP765651
<i>Tantilla armillata</i>	FN256487	Guatemala*	KR814613	KR814644	KR814702	KR814681
<i>Tantilla impensa</i>	FN253542	Guatemala*	KR814614	KR814645	KR814688	KR814677
<i>Tantilla vermiformis</i>	FN256027	Guatemala*	KR814615	KR814646	KR814684	KR814665
<i>Tantilla nigriceps</i>	OMNH41890	USA	--	--	KP765672	KP765655
<i>Tantilla relictal</i>	CAS200845	USA	--	--	AF471045	AF471107
<i>Tantilla relictal2</i>	KW0362	USA	--	--	KP765668	KP765652
<i>Tantilla tjiasmantoi1</i>	CORBIDI:7726	Peru	KY006875	KY006877	--	--
<i>Tantilla tjiasmantoi2</i>	ZFMK:95238	Peru	--	KY006876	--	--
<i>Tantilla melanocephala</i>	MZUSP12976	São Paulo, Brazil	MK209216	MK209331	MK209288	--
<i>Tantilla melanocephala</i>	MNHN 1996.7876	Kourou, French Guiana	AF158424	AF158491	--	--

Table S2 (continued). Species used in this study, vouchers, and GenBank accession numbers. na: not applicable; asterisks (*) indicate locality identity not confirmed.

Species	Ingroup vouchers	Ingroup country	12S rDNA	16S rDNA	Cytb	C-mos
<i>Tantilla melanocephala</i> 1	AMCC101309	Aishalton, Guyana	MT968708	MT968713	MT968722	MT968717
<i>Tantilla melanocephala</i> 2	AMCC101356	Dubulay, Guyana	MT968709	MT968714	MT968723	MT968718
<i>Tantilla melanocephala</i>	UWIZM.2015.18.28	Trinidad	MT968711	MT968716	MT968725	MT968720
<i>Tantilla melanocephala</i>	UWIZM.2016.22.54	Tobago	MT968707	MT968712	MT968721	--
<i>Tantilla melanocephala</i>	MBLUZ 1291	Macuro, Venezuela	MT968710	MT968715	MT968724	MT968719

Table S3. Best partition schemes selected in PartitionFinder for the RaxML and MrBayes analyses, and best models selected in jModeltest for BEAST.

Scheme	Model
PartitionFinder	
12S, 16S rDNA, cytb 1 st codon	GTR+I+G
cytb 2 nd codon	TRN+I+G
cytb 3 rd codon	K81UF+G
c-mos 1 st + 2 nd codon	K80
c-mos 3 rd codon	HKY
jModeltest	
12S rDNA+16S rDNA	TIM2+I+G
cytb	TPM2uf+I+G
c-mos	KHY